Case Docket 21525

SPECIFIC MARKERS FOR PANCREATIC CANCER

Background of the Invention

[0001] The present invention relates to markers for diagnosis of pancreatic cancer comprising at least one polypeptide identified by proteomics to be up-regulated in pancreatic cancer, to an in vitro method for the diagnosis of pancreatic cancer and/or the susceptibility to pancreatic cancer comprising the steps of a) obtaining a biological sample; and b) detecting and/or measuring the increase of specific markers as disclosed herein. Furthermore, screening methods relating to antagonists of the specific markers disclosed herein are provided.

[0002] Pancreatic cancer is a common cause of death in the Western world. It is one of the most aggressive malignant tumors, with an overall 5-year survival rate of 0.4%. In many patients with pancreatic cancer, accurate preoperative diagnosis is difficult to achieve with conventional imaging analyses. Most patients with pancreatic cancer present late in the course of the disease and have either locally extensive or metastatic disease. Overall, only up to 20% are candidates for resection and have the potential for curative surgery. Among the causes for this late presentation is the lack of diagnostic methods for an earlier detection of the disease. Besides this lack of diagnostic methods, the high mortality of patients with pancreatic cancer is additionally caused by a lack of effective treatments. Therefore, the identification of new targets for early diagnosis of pancreatic tumors, and for the development of agents to treat pancreatic cancer is a challenge of paramount importance.

Summary of the Invention

[0003] The problem of identifying polypeptides suitable as markers of pancreatic cancer for early diagnosis of the disease, and the long felt need for such markers, was overcome by the present invention by applying the new technology of proteomics. It was surprisingly found by using proteomic technology that a specific set of polypeptides are differentially expressed

in pancreatic tissue obtained from individuals suffering from pancreatic cancer, as compared to healthy pancreatic tissue. The differentially expressed polypeptides are listed in appended tables 2 and 3. The polypeptides in table 3 are encoded by genes which were previously identified to be up-regulated in pancreatic cancer on the transcriptional level (Iacobuzio-Donahue et al., (2002), Am. J. Pathol. 160, 1239-1249). However, it is well known that regulation on the transcriptional level is not necessarily indicative of a similar regulation of the expression of the respective gene on the translational level. Thus, only by demonstrating that the polypeptides listed in table 3 are up-regulated in pancreatic cancer is it possible to use them for polypeptide-based diagnostic assays for the detection of pancreatic cancer.

[0004] According to one embodiment of the present invention, there is provided a marker for diagnosis of pancreatic cancer comprising at least one polypeptide selected from the group consisting of the polypeptides listed in tables 2, 3 and 6.

[0005] According to another embodiment of the present invention, there is provided an in vitro method for the diagnosis of pancreatic cancer and/or the susceptibility to pancreatic cancer comprising the steps of a) obtaining a biological sample; and b) detecting and/or measuring the increase of at least one marker selected from the polypeptides listed in tables 2, 3 and 6.

[0006] According to a further embodiment of the present invention, there is provided an in vitro method for the diagnosis of pancreatic cancer and/or the susceptibility to pancreatic cancer comprising the steps of a) obtaining a biological sample; and b) detecting and/or measuring the increase of at least one nucleic acid coding for at least one marker selected from the polypeptides listed in tables 2, 3 and 6.

[0007] According to still another embodiment of the present invention, there is provided a screening method for identifying and/or obtaining a compound which interacts with a polypeptide listed in tables 2, 3 and/or 6 whose expression is upregulated in pancreatic cancer, comprising the steps of a) contacting the polypeptide with a compound or a plurality of compounds under conditions which allow interaction of the compound with

the polypeptide; and b) detecting the interaction between the compound or plurality of compounds with the polypeptide.

[0008] According to yet another embodiment of the present invention, there is provided a screening method for identifying and/or obtaining a compound which is an inhibitor of the expression of a polypeptide listed in tables 2, 3 and/or 6 whose expression is upregulated in pancreatic cancer, comprising the steps of a) contacting a host which expresses the polypeptide with a compound; b) determining the expression level and/or activity of the polypeptide; c) determining the expression level and/or activity of the polypeptide in the host as defined in (a), which has not been contacted with the compound; and d) quantitatively relating the expression level of the polypeptide as determined in (b) and (c), wherein a decreased expression level determined in (b) in comparison to (c) is indicative for an inhibitor of the expression of the polypeptide.

[0009] According to yet a further embodiment of the present invention, there is provided a kit for the diagnosis of pancreatic cancer containing one of a) a nucleic acid coding for at least one marker from tables 2, 3 and 6; b) an antibody, or antigen-binding fragment thereof, which binds at least one of the polypeptides listed in tables 2 and 3; or c) a compound that activates or inhibits at least one of the polypeptides listed in tables 2 and 3.

[0010] These and other features, aspects and advantages of the present invention will become better understood with reference to the following description and claims.

Detailed Description of the Invention

[0011] Based on the polypeptides listed in tables 2 and 3, one embodiment of the present invention provides a marker for diagnosis of pancreatic cancer comprising at least one polypeptide selected from the group consisting of the polypeptides listed in tables 2 and/or 3 (SEQ ID NO: 1 to 24 and 26 to 49; and/or SEQ ID NO: 25 and 50 to 55).

[0012] The term "marker" as used herein refers to one or more polypeptides that are regulated in cancer and that can be used to diagnose pancreatic cancer or a susceptibility to

pancreatic cancer either alone or as combinations of multiple polypeptides that are known to be regulated in pancreatic cancer. Preferably, the polypeptides are selected from the group consisting of SEQ. ID NO: 2 to 10, 12 to 15, 17, 19, 20, 23, 24, 27, 28, 31 to 40, 42 to 45, 47 and 48; and/or SEQ ID NO: 25 and 50 to 54. More preferably, the polypeptides are selected from the group consisting of SEQ ID NO: 3, 4, 6, 9, 14, 15, 27, 31 to 35, 37, 39, 40; and/or SEQ ID NO: 50 to 52. Even more preferably, the polypeptides are selected from the group consisting of SEQ ID NO: 4, 6, 9, 14, 15, 31, 33 to 35 and/or SEQ ID NO: 51 and 52. Most preferably, the polypeptides are selected from the group consisting of SEQ ID NO: 4, 6, 14, 15 and 31; and/or SEQ ID NO: 52.

[0013] The term "polypeptide" as used herein, refers to a polymer of amino acids, and not to a specific length. Thus, peptides, oligopeptides and proteins are included within the definition of polypeptide.

[0014] Preferably, the marker of this invention is a marker comprising at least one polypeptide selected from the group consisting of the polypeptides listed in table 2.

[0015] Furthermore, a polypeptide selected from the group consisting of the polypeptides listed in tables 2 and/or 3, may be used as a marker or as part of a marker for diagnosis of pancreatic cancer and/or the susceptibility to pancreatic cancer. Preferably, the polypeptides are selected from the group consisting of SEQ. ID NO: 2 to 10, 12 to 15, 17, 19, 20, 23, 24, 27, 28, 31 to 40, 42 to 45, 47 and 48 from table 2 and/or SEQ ID NO: 25 and 50 to 54 from table 3. These polypeptides are induced at least two fold, as can be seen in tables 2 and 3. More preferably, the polypeptides are selected from the group consisting of SEQ ID NO: 3, 4, 6, 9, 14, 15, 27, 31 to 35, 37, 39, 40 from table 2 and/or SEQ ID NO: 50 to 52 from table 3. These polypeptides are induced at least three fold, as can be seen in tables 2 and 3. Even more preferably, the polypeptides are selected from the group consisting of SEQ ID NO: 4, 6, 9, 14, 15, 31, 33 to 35 from table 2 and/or SEQ ID NO: 51 and 52 from table 3. These polypeptides are induced at least 4 fold, as can be seen in tables 2 and 3. Most preferably, the polypeptides are selected from the group consisting of SEQ ID NO: 4, 6, 14, 15 and 31 from table 2 and/or SEQ ID NO: 52 from table 3, which are the polypeptides that are induced five fold, as shown in tables 2 and 3.

[0016] According to another embodiment, the present invention pertains to a marker for diagnosis of pancreatic cancer comprising at least one polypeptide selected from the group consisting of the polypeptides listed in table 6. Preferably, the at least one polypeptide additionally does not include SEQ ID NO:s 25 and 50 to 55.

[0017] In a preferred embodiment, the marker hereinbefore described additionally comprises at least one of the polypeptides listed in table 5.

[0018] Several groups of polypeptides were identified as markers for pancreatic cancers:

Enzymes

[0019] One of the enzymes that caught our attention was Glutamine γ-glutamyltransferase/tissue transglutaminase (TGLC, SEQ ID NO: 54). It is a member of the transglutaminase family that catalyzes Ca2+ dependent reactions resulting in the post translational modification (cross-linking and conjugation with polyamines) of proteins at the level of glutamine and lysine residues (Greenberg, C. S., Birckbichler, P. J., and Rice, R. H. Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues. FASEB J., *5*: 3071-3077, 1991). Many different roles for this protein have been described, among them apoptosis, adhesion, and differentiation (Amendola, A., Fesus, L., Piacentini, M., and Szondy, Z. "Tissue" transglutaminase in AIDS. J.Immunol.Methods, *265*: 145-159, 2002). There is some controversy on the role of TGLC in apoptosis. While several pieces of evidence suggest that TGLC is a pro-apoptotic protein (Melino, G., et al., Mol.Cell Biol., *14*: 6584-6596, 1994), Jason et al. found that TGLC acts in anti-apoptotic fashion (Boehm, J. E., et al. J.Biol.Chem., *277*: 20127-20130, 2002). Many substrates of TGLC are major extra-cellular matrix (ECM) components such as fibronectin, osteonectin, and collagen, which makes TGLC an important enzyme in ECM development (Raghunath, M., et al., J.Clin.Invest, *98*: 1174-1184, 1996., Nemes, Z., Jr., et al. J.Biol.Chem., *272*: 20577-20583, 1997). Abnormal ECM development is involved in many pathological conditions such as fibrosis and may play a role in the proliferation of fibrous tissue observed in PC. Haroon et al. described that TGLC ECM-promoting abilities are an important part of the host response mechanism against tumor growth (Haroon, Z. A., et al., Lab Invest, *79*: 1679-1686, 1999). Interestingly, loss of TGLC can be a biomarker for prostate adenocarcinoma

(Birckbichler, P. J., et al., Cancer, *89*: 412-423, 2000), which raises the question whether the measured TGLC is produced by neoplastic ductal cells and/or stromal cells. Measurements of mRNA levels in PC, normal tissue and PC cell lines indicate that TGLC is over expressed in both cell types (Iacobuzio-Donahue, C. A., et al., Am.J.Pathol., *160*: 1239-1249, 2002), which would distinguish PC from prostate adenocarcinoma.. Therefore, one preferred embodiment of the present invention is a marker comprising SEQ ID NO: 54.

Cytoskeletal proteins

[0020] Several cytoskeletal proteins were detected at higher levels in PC than in surrounding tissue. One of these is gelsolin (SEQ ID NO: 3), a Ca^{2+} and PIP2 (polyphosphoinositide 4,5-bisphosphate) regulated severing and capping protein, which is a multifunctional actin regulatory protein and has roles in actin remodeling, motility, signaling, apoptosis and cancer (Maruta, H. G proteins cytoskeleton and cancer.Austin, Tex.: R.G. Landes, 1998). In several cancer studies, gelsolin expression has been described as down-regulated during carcinogenesis (breast, colon, stomach, bladder, prostate, and lung) (Asch, H. L., et al., Cancer Res., *56*: 4841-4845, 1996; Dosaka-Akita, H., et al., Cancer Res., *58*: 322-327, 1998, Prasad, S. C., et al. Electrophoresis, *18*: 629-637, 1997). Another example for an up-regulated cytoskeletal protein is fascin (SEQ ID NO: 58), an actin-bundling protein that has a role in cell matrix adhesion, cell interaction and migration. Fascin over expression has been reported in several cancers, such as breast, colon, and ovarian carcinoma (29). The present invention also features fascin 2 as a polypeptide up-regulated in pancreatic cancer (SEQ ID NO: 56). Thus, a preferred embodiment of the present invention is a marker comprising SEQ ID. NO: 3. In another preferred embodiment, the marker comprises SEQ. ID NO: 58. In another preferred embodiment, the marker comprises SEQ. ID NO: 56.

[0021] In our study, cytokeratin 7 (SEQ. ID NO: 52) and cytokeratin 19 (SEQ ID NO: 33) showed strong expression in PC. Both have also been described in other cancers and have been linked with metastasis formation (Moll, R., Int.J.Biol.Markers, *9*: 63-69, 1994.). High protein levels of actinin-4 (SEQ ID NO: 5) were detected in PC. This protein was linked by others with cell motility and cancer invasion (Honda, K., Yamada, T., Endo, R., Ino, Y., Gotoh, M., Tsuda, H., Yamada, Y., Chiba, H., and Hirohashi, S. J.Cell Biol., *140*: 1383-1393,

1998.). Taken together, the apparent strong expression of cytoskeletal proteins is likely to be an important factor in the strong invasiveness and metastasis-forming potential of PC. Thus, a preferred embodiment of the present invention is a marker comprising SEQ ID. NO: 52. In another preferred embodiment, the marker comprises SEQ. ID NO: 33. In another preferred embodiment, the marker comprises SEQ. ID NO: 5.

Metastasis

[0022] Cancer of exocrine pancreas is characterized by extensive local invasion, early lymphatic and hematogenous metastasis. Metastasis in PC has been found in the skeleton, eye, bladder, etc. The extent of angiogenesis depends on the balance between pro-angiogenic or anti-angiogenic factors released from cancer and host cell. Currently, intratumoral microvessel density (IMD) measured by immunocytochemistry appears to be the most reliable parameter for assessing angiogenic activity. Patients with high IMD have decreased survival rates in a variety of cancers (Fujioka, S., et al., Cancer, *92*: 1788-1797, 2001). Thymidine phosphorylase (TYPH or TP, SEQ ID NO: 31) which is identical to platelet-derived endothelial cell growth factor, is strongly expressed in PC and stimulates the chemotaxis of endothelial cells through the 2-deoxy-D-ribose, degradation products of thymidine by TP, thus indirectly inducing angiogenesis (Haraguchi, M., et al. Nature, *368*: 198, 1994.). Shuichi Fujioka et al. found that IMD and TP status were independent predictive indicators for overall as well as relapse-free survival in PC (Fujioka, S., et al., Cancer, *92*: 1788-1797, 2001). An additional protein detected at higher levels in PC than in surrounding tissue likely involved in metastasis formation is osteoblast specific factor 2 (SEQ ID NO:53), a putative bone adhesion protein. Breast carcinoma commonly metastasizes to bone (Guise, T. A. Cancer, *88*: 2892-2898, 2000). Although the role of this protein in PC is not clearly established, our findings suggest a similar role for osteoblast specific factor 2 in PC. Thus, a preferred embodiment of the present invention is a marker comprising SEQ ID. NO: 31. In another preferred embodiment, the marker comprises SEQ. ID NO: 53.

Small GTP-binding proteins

[0023] Four small GTP-binding proteins and interacting proteins were more strongly expressed in PC than in normal pancreas tissue. These include RAN (SEQ. ID NO: 27),

GBLP (guanine nucleotide binding protein β subunit-like protein RACK1, SEQ. ID NO: 47), GDIR (Rho GDP dissociation inhibitor 1, SEQ. ID NO: 55), and IQG1 or IQGAP1 (Ras gtpase activating like protein, SEQ ID NO: 25). Small GTP-binding proteins constitute a superfamily, which is structurally classified into at least five families: the Ras, Rho, Rab, Sar1/Arf, and Ran families and are involved in the regulation of gene expression, cytoskeletal reorganization, and nucleocytoplasmic transport (Takai, Y., et al. Physiol Rev., *81*: 153-208, 2001). RAN is known to enhance androgen receptor-mediated transactivation and was shown to be overexpressed in prostate cancer (Sampson, E. R., et al., J.Biol.Regul.Homeost.Agents, 15: 123-129, 2001). Increased expression of RAN in 81% of prostate tumor cases, may contribute to over proliferation of prostate tumor cells (Li, P., et al., Am J Pathol., 161: 1467-1474,2002). GBLP is an anchoring protein for activated protein kinase Cβ and a variety of other proteins. Protein kinase C plays an important role in angiogenesis and cancer growth. Berns et al. found GBLP up-regulated in during angiogenesis *in vitro* and also associated with nonendothelial cells in angiogenically active tissue (Berns, H., et al., FASEB J., *14*: 2549-2558, 2000). Further more, mRNA expression of GBLP is detected in epithelial cells of human colon carcinoma and proliferating epithelial cell of normal colon tissue. Therefore, there is a likely link between high GBLP expression and tumor growth. GDIR (Rho GDP dissociation inhibitor) had been found up-regulated in a chemoresistant fibrosarcoma cell line by 2D-PAGE (Sinha, P., et al., Electrophoresis, *20*: 2961-2969, 1999) and may block apoptotic signal pathway mediated by Ras and c-jun kinase, resulting in the increase resistance against environmental stress. IQG1 (Ras GTPase-activating-like protein) is a widely expressed 190-kDa Cdc42-, Rac1-, and calmodulin-binding protein that interacts with F-actin in vivo and that can cross-link F-actin microfilaments in vitro. IQG1 negatively regulates the Ecc-based (E-cadherin/catenin complex) cell-cell adhesion by dissociating alpha-catenin. Up-regulation of IQGAP1 is correlated with the malignant phenotype in gastric cancer (Sugimoto, N., et al., J. Hum. Genet., 46: 21-25, 2001). By immunohistochemical analysis, IQGAP1 was found overexpressed in colorectal carcinoma and associated with carcinoma invasion (Nabeshima, K., et al., Cancer Lett., *176*: 101-109, 2002). Since cancer invasiveness is associated with the localized disruption of cell-cell adhesion, both our results and Iacobuzio-Donahue et al.'s data suggest that IQGAP1 may be involved in the disruption of local adhesion and in PC invasion to surrounding tissue. Thus, a preferred embodiment of the present invention is a

marker comprising SEQ ID NO: 27. In another preferred embodiment, the marker comprises SEQ. ID NO: 47. In another preferred embodiment, the marker comprises SEQ. ID NO: 55. In another preferred embodiment, the marker comprises SEQ. ID NO: 25.

S100 protein family

[0024] Another protein with high-level expression in PC is S109 (S100A9, MRP-14, calgranulin B, SEQ ID NO: 49), a member of the S100 protein family of highly homologous low molecular weight calcium binding proteins. Calgranulins are characterized by cell type-specific expression in cells of epithelial, myeloid and endothelial origin and accumulation at sites of acute and chronic inflammation (e.g. rheumatoid arthritis, cystic fibrosis, psoriasis, allergic dermatitis, inflammatory bowel diseases) (Donato, R. Int.J.Biochem.Cell Biol., *33*: 637-668, 2001). S100A8 and S100A9 can form a noncovalent heterodimer protein complex called calprotectin. Current reports support that both of S100A9 and S100 A8 have wide range of possible intracellular as well as extracellular functions (Schafer, B. W. and Heizmann, C. W. Trends Biochem.Sci., *21*: 134-140, 1996). S100A8 and S100A9 are negatively regulated by glucocorticoids in a c-Fos-dependent manner and over expressed throughout skin carcinogenesis (Gebhardt, C., et al., Oncogene, *21*: 4266-4276, 2002). These proteins are also more strongly expressed in colorectal carcinoma than in matched normal colon mucosa, as shown by proteomics analysis (Stulik, J., et al., Electrophoresis, *20*: 1047-1054, 1999). S100A9 has been detected in cultured human adenocarcinoma (AC) cells derived from various organs, and is associated with tumor differentiation in pulmonary adenocarcinoma (Arai, K., et al., Oncol.Rep., *8*: 591-596, 2001). Iacobuzio-Donahue et al's work indicates that over expression of S100A4 in PC is associated with poor differentiation and DNA hypomethylation (Rosty, C., et al., Am.J.Pathol., *160*: 45-50, 2002). Thus, a preferred embodiment of the present invention is a marker comprising SEQ ID. NO: 49.

Annexin

[0025] We found that annexin 1 (SEQ ID NO: 51) and annexin 2 (SEQ ID NO: 19) have high level of expression in PC. Both are members of a family of Ca^{2+}-dependent membrane-binding proteins. Described functions include, among others, an important role in malignant transformation (Masaki, T., et al., Hepatology, *24*: 72-81, 1996), the control of

epithelial cell line proliferation (Solito, E., et al., Cell Growth Differ., *9*: 327-336, 1998), and mediation of apoptosis (Canaider, S., et al.,. Life Sci., *66*: L265-L270, 2000). Evidence in support of causative roles for any annexins in the development of cancer is still mainly circumstantial. In MCF-7 breast carcinoma cells, overexpression of annexin1 led to abrogation of Ca2+ release after activation of purinergic or bradykinin receptors (Frey, B. M., et al., FASEB J., *13*: 2235-2245, 1999), while over expression of annexin1 in rat 2 fibroblasts leads to direct inhibition of cytosolic PLA2, which in turn depresses the serum response element of c-fos (Oh, J., et al., FEBS Lett., *477*: 244-248, 2000). Collectively, these studies imply a growth-suppressive role for annexin1. These results are not supported by the finding that annexin 1 is strongly up-regulated in a prostate cancer cell line (Vaarala, M. H., Lab Invest, *80*: 1259-1268, 2000), esophageal cancer (Emmert-Buck, M. R., et al., Mol.Carcinog., *27*: 158-165, 2000), a stomach cancer cell line (Sinha, P., et al., J.Biochem.Biophys.Methods, *37*: 105-116, 1998), mammary adenocarcinoma (Pencil, S. D. and Toth, M. Clin.Exp.Metastasis, *16*: 113-121, 1998), and hepatocarcinoma (de Coupade, C., et al., Hepatology, *31*: 371-380, 2000). In hepatocarcinoma, study also showed that the proliferative rate of both normal and malignant hepatocytes was attenuated by antisense to annexin 1. These and our data suggest that cell growth is associated with elevated rather than reduced levels of annexin 1, which is also supported by the studies of Iacobuzio-Donahue et al. (Am. J. Pathol., *160*: 1239-1249, 2002). Thus, a preferred embodiment of the present invention is a marker comprising SEQ ID NO: 51. In another preferred embodiment, the marker comprises SEQ. ID NO: 19.

[0026] Some additional proteins highly expressed in PC may have either clear roles in PC or an indirect link with PC, e.g. BGH3 (TGF-β1-induced protein, SEQ ID NO: 6) which is a secretory protein and acts as a marker for biologically active TGF- β 1 (Langham, R. G., et al., Transplantation, *72*: 1826-1829, 2001). Thus, a preferred embodiment of the present invention is a marker comprising SEQ ID NO: 6.

[0027] With the identification of polypeptides regulated in pancreatic cancer, the present invention provides an in vitro method for the diagnosis of pancreatic cancer and/or the susceptibility to pancreatic cancer comprising the steps of obtaining a biological sample; and detecting and/or measuring the increase of a marker described hereinbefore.

[0028] The term "detection" as used herein refers to the qualitative determination of the absence or presence of polypeptides.

[0029] The term "measured" as used herein refers to the quantitative determination of the differences in expression of polypeptides in biological samples from patients with pancreatic cancer and biological samples from healthy individuals.

[0030] Methods for detection and/or measurement of polypeptides in biological samples are well known in the art and include, but are not limited to, Western-blotting, ELISAs or RIAs. Antibodies recognizing the polypeptides listed in table 2, 3, 5 and/or 6 can either be generated for the purpose of detecting the polypeptides, eg. by immunizing rabbits with purified proteins, or known antibodies recognizing the polypeptides can be used. For example, an antibody capable of binding to the denatured proteins, such as a polyclonal antibody, can be used to detect the peptides of this invention in a Western Blot. An example for a method to measure a marker is an ELISA. This type of protein quantitation is based on an antibody capable of capturing a specific antigen, and a second antibody capable of detecting the captured antigen. A further method for the detection of a diagnostic marker for pancreatic cancer is by analyzing biopsy specimens for the presence or absence of the markers of this invention. Methods for the detection of these markers are well known in the art and include, but are not limited to, immunohistochemistry or immunofluorescent detection of the presence or absence of the polypeptides of the marker of this invention. Methods for preparation and use of antibodies, and the assays mentioned hereinbefore are described in Harlow, E. and Lane, D. Antibodies: A Laboratory Manual, (1988), Cold Spring Harbor Laboratory Press.

[0031] The accuracy of the diagnosis of pancreatic cancer can be increased by analyzing combinations of multiple polypeptides listed in tables 2, 3, 5 and/or 6. Thus, the in vitro method herein before described, comprises a marker which comprises at least two, preferably at least three, more preferably at least four, even more preferably at least five, and most preferably at least six of the polypeptides listed in table 2,3, 5 and/or 6.

[0032] For diagnosis of pancreatic cancer, suitable biological samples need to be analyzed for the presence or absence of a marker. The biological samples can be serum, plasma, pancreatic juice or cells of pancreatic tissue. Cells from pancreatic tissue can be obtained by ERCP, secretin stimulation, fine-needle aspiration, cytologic brushings and large-bore needle biopsy.

[0033] It is also possible to diagnose pancreatic cancer by detecting and/or measuring nucleic acid molecules coding for the marker hereinbefore described. Preferably, the nucleic acid molecule is RNA or DNA. In another embodiment, the DNA is a cDNA.

[0034] In one embodiment of the present invention, the in vitro method herein before described comprises comparing the expression levels of at least two of the nucleic acids encoding the polypeptides in an individual suspected to suffer from pancreatic cancer and/or to be susceptible to pancreatic cancer, to the expression levels of the same nucleic acids in a healthy individual.

[0035] In another embodiment of the present invention the in vitro method herein before described comprises comparing the expression level of the marker in an individual suspected to suffer from pancreatic cancer and/or to be susceptible to pancreatic cancer to the expression levels of the same marker in a healthy individual. In a more preferred embodiment of the in vitro method, an increase or decrease of the expression levels of the marker is indicative of pancreatic cancer or the susceptibility to pancreatic cancer.

[0036] The present invention also provides a screening method for identifying and/or obtaining a compound which interacts with a polypeptide listed in table 2 and/or 3 whose expression is upregulated in pancreatic cancer, comprising the steps of contacting the polypeptide with a compound or a plurality of compounds under conditions which allow interaction of the compound with the polypeptide; and detecting the interaction between the compound or plurality of compounds with the polypeptide.

[0037] The "interaction" in the screening methods as disclosed herein may be measured by conventional methods. The type of conventional method for testing the interaction of a

compound with a polypeptide that is soluble, as opposed to membrane associated, can be an in vitro method using either purified recombinant polypeptide, or native polypeptide purified from cells that endogenously express the polypeptide. As a non-limiting example, a polypeptide of the invention can be bound to beads or immobilized on plastic or other surfaces, and interaction of a compound with the polypeptide can be measured by either using a labeled compound and measuring the label bound to the polypeptide or by displacement of a labeled known ligand from the polypeptide.

[0038] For polypeptides that are associated with the cell membrane on the cell surface, or which are expressed as transmembrane or integral membrane polypeptides, the interaction of a compound with the polypeptides can be detected with different methods which include, but are not limited to, methods using cells that either normally express the polypeptide or in which the polypeptide is overexpressed, eg. by detecting displacement of a known ligand which is labeled by the compound to be screened. Alternatively, membrane preparations may be used to test for interaction of a compound with such a polypeptide

[0039] Interaction assays to be employed in the method disclosed herein may comprise FRET-assays (fluorescence resonance energy transfer; as described, inter alia, in Ng, Science 283 (1999), 2085-2089 or Ubarretxena-Belandia, Biochem. 38 (1999), 7398-7405), TR-FRETs and biochemical assays as disclosed herein. Furthermore, commercial assays like "Amplified Luminescent Proximity Homogenous Assay^{TM}" (BioSignal Packard) may be employed. Further methods are well known in the art and, inter alia, described in Fernandez, Curr. Opin. Chem. Biol. 2 (1998), 547-603.

[0040] The "test for interaction" may also be carried out by specific immunological and/or biochemical assays which are well known in the art and which comprise, e.g., homogenous and heterogenous assays as described herein below. The interaction assays employing read-out systems are well known in the art and comprise, inter alia, two-hybrid screenings (as, described, inter alia, in EP-0 963 376, WO 98/25947, WO 00/02911; and as exemplified in the appended examples), GST-pull-down columns, co-precipitation assays from cell extracts as described, inter alia, in Kasus-Jacobi, Oncogene 19 (2000), 2052-2059, "interaction-trap" systems (as described, inter alia, in US 6,004,746) expression cloning

(e.g. lamda gt11), phage display (as described, inter alia, in US 5,541,109), in vitro binding assays and the like. Further interaction assay methods and corresponding read out systems are, inter alia, described in US 5,525,490, WO 99/51741, WO 00/17221, WO 00/14271 or WO 00/05410. Vidal and Legrain (1999) in Nucleic Acids Research 27, 919-929 describe, review and summarize further interaction assays known in the art which may be employed in accordance with the present invention.

[0041] Homogeneous (interaction) assays comprise assays wherein the binding partners remain in solution and comprise assays, like agglutination assays. Heterogeneous assays comprise assays like, inter alia, immuno assays, for example, Enzyme Linked Immunosorbent Assays (ELISA), Radioactive Immunoassays (RIA), Immuno Radiometric Assays (IRMA), Flow Injection Analysis (FIA), Flow Activated Cell Sorting (FACS), Chemiluminescent Immuno Assays (CLIA) or Electrogenerated Chemiluminescent (ECL) reporting.

[0042] The present invention further provides a screening method for identifying and/or obtaining a compound which is an inhibitor or an antagonist of a polypeptide listed in table 2 and/or 3 whose expression is upregulated in pancreatic cancer, comprising the steps of a) contacting the polypeptide with a compound identified and/or obtained by the screening method described above under conditions which allow interaction of the compound with the polypeptide; b) determining the activity of the polypeptide; c) determining the activity of the polypeptide expressed in the host as defined in (a), which has not been contacted with the compound; and d) quantitatively relating the activity as determined in (b) and (c), wherein a decreased activity determined in (b) in comparison to (c) is indicative for an inhibitor or antagonist. The terms inhibitors and antagonists as used herein are used interchangeably. This screening assay can be performed either as an in vitro assay, or as a host-based assay. The host to be employed in the screening methods of the present invention and comprising and/or expressing a polypeptide listed in table 2, 3, 5 and/or 6 may comprise prokaryotic as well as eukaryotic cells. The cells may comprise bacterial cells, yeast cells, as well as cultured (tissue) cell lines, inter alia, derived from mammals. Furthermore animals may also be employed as hosts, for example an non-human transgenic animal. Accordingly, the host (cell) may be transfected or transformed with the vector comprising a nucleic acid molecule coding for a polypeptide which is differentially

regulated in pancreatic cancer as disclosed herein. The host cell or host may therefore be genetically modified with a nucleic acid molecule encoding such a polypeptide or with a vector comprising such a nucleic acid molecule.

[0043] The term "genetically modified" means that the host cell or host comprises in addition to its natural genome a nucleic acid molecule or vector coding for a polypeptide listed in table 2, 3, 5 and/or 6 or at least a fragment thereof. The additional genetic material may be introduced into the host (cell) or into one of its predecessors/parents. The nucleic acid molecule or vector may be present in the genetically modified host cell or host either as an independent molecule outside the genome, preferably as a molecule which is capable of replication, or it may be stably integrated into the genome of the host cell or host.

[0044] As mentioned herein above, the host cell of the present invention may be any prokaryotic or eukaryotic cell. Suitable prokaryotic cells are those generally used for cloning like E. coli or Bacillus subtilis. Yet, these prokaryotic host cells are also envisaged in the screening methods disclosed herein. Furthermore, eukaryotic cells comprise, for example, fungal or animal cells. Examples for suitable fungal cells are yeast cells, preferably those of the genus Saccharomyces and most preferably those of the species Saccharomyces cerevisiae. Suitable animal cells are, for instance, insect cells, vertebrate cells, preferably mammalian cells, such as e.g. CHO, HeLa, NIH3T3 or MOLT-4. Further suitable cell lines known in the art are obtainable from cell line depositories, like the American Type Culture Collection (ATCC).

[0045] The hosts may also be selected from non-human mammals, most preferably mice, rats, sheep, calves, dogs, monkeys or apes. As described herein above, the animals/mammals also comprise non-human transgenic animals, which preferably express at least one polypeptide differentially regulated in pancreatic cancer as disclosed herein. Preferably, the polypeptide is a polypeptide which is up-regulated in tissue derived from patients with pancreatic cancer. Yet it is also envisaged that non-human transgenic animals be produced which do not express marker genes as disclosed herein or who express limited amounts of the marker gene products. The animals are preferably related to polypeptides which are down-regulated in pancreatic cancer. Transgenic non-human animals comprising and/or

expressing the up-regulated polypeptides of the present invention or alternatively, which comprise silenced or less efficient versions of down-regulated polypeptides are useful models for studying the development of pancreatic cancer and provide for useful models for testing drugs and therapeutics for pancreatic cancer treatment and/or prevention.

[0046] A compound which interacts with a polypeptide listed in table 2, 3, 5 and/or 6 and which inhibits or antagonizes the polypeptide is identified by determining the activity of the polypeptide in the presence of the compound.

[0047] The term "activity" as used herein relates to the functional property or properties of a specific polypeptide. For the enzymes listed in table 2, 3, 5 and/or 6, the term "activity" relates to the enzymatic activity of a specific polypeptide. Activity assays for the enzymes listed in table 2, 3, 5 and/or 6 are well known.

[0048] For adhesion molecules listed in table 2, 3, 5 and/or 6, the term "activity" relates to the adhesive properties of a polypeptide and may be determined using assays such as, but not limited to, adhesion assays, cell spreading assays, or in vitro interaction of the adhesion molecule with a known ligand. Such assays are well known in the art.

[0049] For cytoskeletal proteins, the term "activity" relates to the regulation of the cytoskeleton by such polypeptides, or to their incorporation into the cytoskeleton. As a non-limiting example, the ability of Gelsolin to regulate actin polymerization, or of Filamin A to promote orthogonal branching of actin filaments, may be determined using in vitro actin polymerization assays. Activity in relation to the regulation of cytoskeletal structures may further be determined by, as non-limiting examples, cell spreading assays, cell migration assays, cell proliferation assays or immunofluorecence assays, or by staining actin filaments with fluorescently labeled phalloidin. All of these assays are well known to the person skilled in the art.

[0050] For ion channels (Chloride intracellular channel protein) the term "activity" relates to ion flux (Chloride lux) across the membrane. Methods to determine ion flux across membranes are well known to the person skilled in the art.

[0051] For transcription factors, eg. KIAA 1034, the term "activity" relates to their ability to regulate gene transcription. The transcriptional activity of a polypeptide can be determined using commonly used assays, such as a reporter gene assay.

[0052] For growth factors and hormones or their receptors, the term "activity" relates to their ability to bind to their receptors or ligands, respectively, and to induce receptor activation and subsequent signaling cascades, and/or it relates to the factor's or receptor's ability to mediate the cellular function or functions eventually caused by growth factor or hormone mediated receptor activation. Growth factor or hormone binding to receptors can be determined by commonly known ligand binding assays. Receptor activation can be determined by testing for receptor auto-phosphorylation, or by assaying for modification or recruitment of downstream signaling mediators to the receptors (by immunoprecipitation and Western Blotting of signaling complexes). Cellular functions regulated by growth factors or hormones and their receptors can be cell proliferation (eg determined by using thymidine incorporation or cell counts), cell migration assays (eg determined by using modified Boyden chambers), cell survival or apoptosis assays (eg determined by using DAPI staining), angiogenesis assays (eg in vitro assays to measure endothelial tube formation that are commercially available). In addition to these assays, other assays may be used as well to determine these and other cellular functions.

[0053] Inhibitors or antagonists of a polypeptide listed in tables 2 and/or 3 are identified by the screening method described above when there is a decreased activity determined in the presence of the compound in comparison to the absence of the compound in the screening method, which is indicative for an inhibitor or antagonist.

[0054] Further to the screening methods disclosed above, this invention provides a screening method for identifying and/or obtaining a compound which is an inhibitor of the expression of a polypeptide listed in tables 2 and/or 3 whose expression is upregulated in pancreatic cancer, comprising the steps of a) contacting a host which expresses the polypeptide with a compound; b) determining the expression level and/or activity of the polypeptide; c) determining the expression level and/or activity of the polypeptide in the host as defined in (a), which has not been contacted with the compound; and d)

quantitatively relating the expression level of the polypeptide as determined in (b) and (c), wherein a decreased expression level determined in (b) in comparison to (c) is indicative for an inhibitor of the expression of the polypeptide.

[0055] An inhibitor of the expression of a polypeptide listed in table 2, 3, 5 and/or 6 is identified by the screening method described hereinbefore when a decreased expression of the protein is determined in the presence of the compound in comparison to the absence of the compound in the screening method, which is indicative for an inhibitor of expression of a polypeptide.

[0056] The term "express" as used herein relates to expression levels of a polypeptide listed in table 2, 3, 5 and/or 6 which is up-regulated in pancreatic cancer, in cells, preferably in a pancreatic adenocarcinoma cell line, which are elevated as compared to the expression levels of the same polypeptide in healthy pancreatic cells. Preferably, expression levels are at least 2 fold, more preferably at least 3 fold, even more preferably at least 4 fold, most preferably at least 5 fold higher than in healthy pancreatic cells.

[0057] Furthermore, the present invention provides a compound identified and/or obtained by any of the screening methods hereinbefore described. The compound is further comprised in a pharmaceutical composition. A method for the preparation of the pharmaceutical composition comprising formulating the compound in a pharmaceutically acceptable carrier or diluent is also claimed. Any conventional carrier material can be utilized. The carrier material can be an organic or inorganic one suitable for eteral, percutaneous or parenteral administration. Suitable carriers include water, gelatin, gum arabic, lactose, starch, magnesium stearate, talc, vegetable oils, polyalkylene-glycols, petroleum jelly and the like. Furthermore, the pharmaceutical preparations may contain other pharmaceutically active agents. Additional additives such as flavoring agents, stabilizers, emulsifying agents, buffers and the like may be added in accordance with accepted practices of pharmaceutical compounding.

[0058] The compound may be used for the preparation of a medicament for the treatment or prevention of pancreatic cancer. In addition, the compound may also be used for the

preparation of a diagnostic composition for diagnosing pancreatic cancer or a predisposition for pancreatic cancer. Preferably, the compound comprises an antibody, an antibody-derivative, an antibody fragment, a peptide or an antisense construct.

[0059] Within the scope of the present invention, antibodies against the proteins listed in tables 2 and/or 3, or antigen-binding fragments thereof, may be used in an in vitro method for the diagnosis of pancreatic cancer.

[0060] In order to efficiently perform diagnostic screenings, the present invention provides a kit for the diagnosis of pancreatic cancer comprising one or more of the antibodies, or antigen-binding fragments thereof, described above. Another kit provided by this invention is a kit for the diagnosis of pancreatic cancer comprising one or more of the nucleic acids coding for the marker hereinbefore described. Yet another kit provided by this invention is a kit for screening of compounds that antagonize any of the polypeptides listed in tables 2 and/or 3 or inhibit the expression of any of the polypeptides.

[0061] The present invention pertains to a marker for diagnosis of pancreatic cancer comprising at least one polypeptide selected from the group consisting of the polypeptides listed in table 6. Preferably, the marker does not include SEQ ID NO:s 25 and 50 to 55. In a more preferred embodiment, the marker comprises at least one of the polypeptides listed in table 5.

[0062] The present invention also provides an in vitro method for the diagnosis of pancreatic cancer and/or the susceptibility to pancreatic cancer comprising the steps of

a) obtaining a biological sample; and
b) detecting and/or measuring the increase of at least one of the polypeptides listed in table 6.

[0063] Preferably, the in vitro method additionally comprises the step of detecting and/or measuring the decrease of at least one of the polypeptides listed in table 5. More preferably, in the in vitro method, the at least one polypeptide does not include SEQ ID NO:s 25 and

50 to 55. Even more preferably, in the in vitro method, the biological sample is derived from the group consisting of serum, plasma, pancreatic juice and cells of pancreatic tissue.

[0064] The present invention further provides an in vitro method for the diagnosis of pancreatic cancer and/or the susceptibility to pancreatic cancer comprising the steps of

a) obtaining a biological sample; and

b) detecting and/or measuring the increase of at least one nucleic acid coding for the marker hereinbefore described.

[0065] Preferably, in the in vitro method, the nucleic acid molecule is RNA or DNA. More preferably, in the in vitro method, the DNA is a cDNA.

[0066] In a further more preferred embodiment of any of the in vitro methods hereinbefore described, the expression levels of at least one of the nucleic acids in an individual suspected to suffer from pancreatic cancer and/or to be susceptible to pancreatic cancer is compared to the expression levels of the same nucleic acids in a healthy individual. In a most preferred embodiment of any of the in vitro methods hereinbefore described, the expression level of the marker in an individual suspected to suffer from pancreatic cancer and/or to be susceptible to pancreatic cancer is compared to the expression levels of the same marker in a healthy individual.

[0067] In a further preferred embodiment of the in vitro method hereinbefore described, an increase of the expression levels of the marker is indicative of pancreatic cancer or the susceptibility to pancreatic cancer.

[0068] The present invention also pertains to a screening method for identifying and/or obtaining a compound which interacts with a polypeptide selected from the group consisting of the polypeptides listed in table 6 whose expression is upregulated in pancreatic cancer, comprising the steps of

a) contacting the polypeptide with a compound or a plurality of compounds under conditions which allow interaction of the compound with the polypeptide; and

b) detecting the interaction between the compound or plurality of compounds with the polypeptide.

[0069] Furthermore, the present invention provides a screening method for identifying and/or obtaining a compound which is an inhibitor or an antagonist of a polypeptide listed in table 6 whose expression is upregulated in pancreatic cancer, comprising the steps of

a) contacting the polypeptide with a compound identified and/or obtained by the screening method of claim 39 under conditions which allow interaction of the compound with the polypeptide;
b) determining the activity of the polypeptide;
c) determining the activity of the polypeptide expressed in the host as defined in (a), which has not been contacted with the compound; and
d) quantitatively relating the activity as determined in (b) and (c), wherein a decreased activity determined in (b) in comparison to (c) is indicative for an inhibitor or antagonist.

[0070] The present invention also provides a screening method for identifying and/or obtaining a compound which is an inhibitor of the expression of a polypeptide selected from the group consisting of the polypeptides listed in table 6 whose expression is upregulated in pancreatic cancer, comprising the steps of

a) contacting a host which expresses the polypeptide with a compound,
b) determining the expression level and/or activity of the polypeptide;
c) determining the expression level and/or activity of the polypeptide in the host as defined in (a), which has not been contacted with the compound; and
d) quantitatively relating the expression level of the polypeptide as determined in (b) and (c), wherein a decreased expression level determined in (b) in comparison to (c) is indicative for an inhibitor of the expression of the polypeptide.

[0071] The present invention provides a compound identified and/or obtained by the screening methods hereinbefore described.

[0072] In addition, the present invention provides a pharmaceutical composition comprising the compound hereinbefore described. Also provided is a method for the preparation of the pharmaceutical composition hereinbefore described comprising formulating the compound hereinbefore described in a pharmaceutically acceptable carrier or diluent.

[0073] The present invention provides a use of a compound hereinbefore described for the preparation of a medicament for the treatment or prevention of pancreatic cancer. Also provided is a use of a compound hereinbefore described for the preparation of a diagnostic composition for diagnosing pancreatic cancer or a predisposition for pancreatic cancer. In a preferred embodiment, the uses hereinbefore described relate to a compound comprising an antibody, an antibody-derivative, an antibody fragment, a peptide or an antisense construct.

[0074] Within the scope of the present invention, antibodies against the proteins listed in tables 5 and/or 6, or antigen-binding fragments thereof, may be used in an in vitro method for the diagnosis of pancreatic cancer.

[0075] In order to efficiently perform diagnostic screenings, the present invention provides a kit for the diagnosis of pancreatic cancer comprising one or more of the antibodies, or antigen-binding fragments thereof, described above. Another kit provided by this invention is a kit for the diagnosis of pancreatic cancer comprising one or more of the nucleic acids coding for the marker hereinbefore described. Yet another kit provided by this invention is a kit for screening of compounds that antagonize any of the polypeptides listed in tables 5 and/or 6 or inhibit the expression of any of the polypeptides.

[0076] In the present invention, the proteins, compounds, kits, methods and uses substantially as herein before described, especially with reference to the foregoing examples are also claimed.

Examples:

Collection of tissue samples

[0077] Pancreatic carcinomas and adjacent tissue were collected from the patients listed in table 1. Samples were collected shortly after the resection (less than 30 minutes), and fast frozen in liquid nitrogen for about 1 minute, then stored in a freezer at a temperature of –80°C.

Characterization of formalin-fixed specimens

[0078] Histopathological characterization was carried out by using hematoxylin-eosin-stained sections of formalin-fixed and paraffin-embedded specimens. Tumors were classified using the WHO system. The types of pancreatic carcinomas included in the study are shown in table 1.

[0079] The twelve pancreatic carcinoma samples used in this study were ductal carcinomas which constitute the overwhelming proportion of pancreatic carcinomas. The patient-matched samples from histologically normal tissue surrounding the carcinoma were used as controls. We carried out 12 pairs of 2-dimensional electrophoresis maps for comparing protein expression between tumor tissue and normal control tissue. For protein identification, the samples were pooled, thus generating pan-Carcinoma and pan-Normal protein extracts. Quantification was carried out in two steps: (I) Gels from the pooled samples were compared using the PDQuest image analysis software. (II) The changes identified at the level of the pooled samples were cross-validated by an analysis of the individual samples. The change factors shown in table 2, 3, 5 and 6 were determined using the pooled samples.

Preparation of samples for electrophoresis

[0080] Samples cleaned of clots and contaminating tissue were frozen in liquid nitrogen, then ground to powder. Samples were suspended in lysis buffer (8M urea, 4% CHAPS, 40mMol/L Tris-Cl, 0.5% carrier amphollytes, 100mMol/L DTT and 0.1ìg/il PMSF) and centrifuged at 12000rpm for 30 minutes. The supernatants were stored at –80°C. The

protein concentration in the extracts was determined by the Bradford method (Bradford, M. Anal. Biochem. 72, 248 (1976).

Two-dimensional gel electrophoresis

[0081] Samples containing 1 mg of protein were loaded onto the rehydrated IPG strip (18 cm, pH3~10) by using the cup loading method. IEF was performed using Pharmacia Multiphor apparatuses under the following conditions: First, the voltage was increased 200V-5000V over 24hrs, then a constant voltage of 5000V was applied for 24 hrs, the running temperature was 20°C. After IEF, the strips were equilibrated with 10 ml equilibration solution I (6 M Urea, 50 mM Tris pH 8.8, 30 % Glycerol, 2.0 % SDS, 30 mM Dithioerythritol) for 15 min, then for another 15 min with equilibration solution II (6 M Urea, 50 mM Tris pH 8.8, 30 % Glycerol, 2.0 % SDS, 0.23 M Iodoacetamide).

[0082] The second dimension SDS polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using a Hoefer ISO_DALT apparatus (10 gels/run, 24×20 cm), IEF strips were loaded onto 12% homogeneous polyacrylamide gels (1.5 mm x 24 cm × 20 cm). The gels were run in TGS_Buffer (250 mM Tris, 1.92 M Glycine, 1% (w/v) SDS, pH = 8.3, Bio-Rad) at a constant voltage (80 V, 20°C).

Gel fixation and staining

[0083] Gels were fixed in 50% Methanol/20% acetic acid for 30 min, then washed in ultra-pure water for 30 min and stained with NOVEX Colloidal Blue staining Kit (Invitrogen) following the manufacturer's recommendations.

Protein Identification

[0084] The protein identification was performed using a two-step procedure.

In-gel digestion

[0085] Spots were picked and transferred into 96-well by a spot picking robot. From each gel, 600-800 spots were picked. The spots were destained with 100μl of 30% acentonitrile in 50Mm ammonium bicarbonate, washed in ultra pure-water and dried in a speed vac evaporator. The dry gel pieces were digested with 10ng/μl trypsin (Promega, Madison,

USA) solution in 500 nM ammonium bicarbonate at room temperature for 16 h maximum. The peptides from each spot were extracted with 20µl of 0.1% trifluore acetic acid (TFA) in 50% acetonitrile. The matrix solution consisted of 0.025%(w/v) alfa-cyano-4-hydroxy cinammic acid (Sigma) in 50% acetonitrile/0.1% TFA with internal standard peptides des-Arg-Bradykinin(Sigma, MW 904.4681 Da) and adrenocorticotropic hormone fragment 18-39 (Sigma, MW 2465.1989 Da).

Analysis by MALDI-TOF

[0086] 1.5ul of peptide extract and 1.0µl of matrix solution were stimulaneously applied to the spots on the MS target. Recrystallization was carried out as specified by the instruments manufacturer. The samples were analyzed in a MALDI-time of flight Mass spectrometer (Autoflex, Bruker Analytics, Bremen, Germany). Peak annotation and database search by peptide matching was performed by in house developed software. The peptide mass was compared with theoretic peptide masses of all available proteins from all species. The monoisotopic mass was used and a mass tolerance of 0.0025% was allowed. 4 matching peptides were the minimal requirement for an identity assignment. Mismatch or miscleavage sites were not considered.

Table 1: Clinical and histopathological characteristics of samples

NO: of Samples	Sex	Age	Tumor location	Histology	Metastasis in lymph nodes
PC-01	Male	48	Head of pancreas	Middle differentiated ductal adenocarcinoma	Yes
PC-02	Male	68	Head of pancreas	Poorly differentiated adenocarcinoma	Yes
PC-03	Male	44	Head of pancreas	Poorly differentiated ductal adenocarinoma, clear cell type	Yes
PC-04	Male	66	Head of pancreas	Well differentiated ductal adenocarcinoma	Yes
PC-05	Female	45	Head of pancreas	Well differentiated ductal adenocarcinoma	No
PC-06	Female	65	Head of pancreas	Well differentiated ductal adenocarcinoma	Yes
PC-07	Male	59	Head of pancreas	Middle differentiated ductal adenocarcinoma	Yes
PC-08	Female	62	Body of pancreas	Well differentiated ductal adenocarcinoma	Yes
PC-09	Male	54	Head of pancreas	Middle differentiated ductal adenocarcinoma	No
PC-10	Female	53	Head of pancreas	Well differentiated ductal adenocarcinoma	No
PC-11	Female	54	Head of pancreas	Middle differentiated ductal adenocarcinoma	Yes
PC-12	Female	69	Head of pancreas	Middle differentiated ductal adenocarcinoma	Yes

Table 2: Proteins up-regulated in pancreatic cancer I

Protein	Acc No	Description	SEQ ID NO:	Fold Change
sw:CATD_HUMAN	P07339	Cathepsin D precursor (ec 3.4.23.5).	1	<2
sw:IDHC_HUMAN	O75874	Isocitrate dehydrogenase [NADP] cytoplasmic (ec 1.1.1.42)	2	2
sw:GELS_HUMAN	P06396	Gelsolin precursor, plasma	3	3
sw:CFAB_HUMAN	P00751	Complement factor B precursor (ec 3.4.21.47)	4	5
sw:AAC4_HUMAN	O43707	Alpha-actinin 4 (non-muscle alpha-actinin 4)	5	2
sw:AAC1_HUMAN	P12814	Alpha-actinin 1 (alpha-actinin cytoskeletal isoform)	7	2
sw:TBA4_HUMAN	P05215	Tubulin alpha-4 chain.	8	2
sw:ABP2_HUMAN	P21333	Filamin A (Endothelial actin-binding protein)	9	4
sw:TAGL_HUMAN	P37802	Transgelin 2 (smooth muscle protein 22-alpha)	10	2
sw:TPM4_HUMAN	P07226	Tropomyosin alpha 4 chain	11	<2
sw:BGH3_HUMAN	Q15582	Transforming growth factor-beta induced protein IG-H3 precursor	6	5
sw:CALD_HUMAN	Q05682	Caldesmon (cdm)	12	2
sw:ENOL_HUMAN	Q05524	Alpha enolase	13	2
sw:ACY1_HUMAN	Q03154	Aminoacylase-1	14	5
sw:CAPB_HUMAN	P47756	F-actin capping protein beta subunit (capz beta)	15	5
sw:IPYR_HUMAN	Q15181	Inorganic pyrophosphatase	16	<2
sw:LEG3_HUMAN	P17931	Galectin-3 (galactose-specific lectin 3).	17	2
sw:POR2_HUMAN	P45880	Voltage-dependent anion-selective channel protein 2	18	<2
SW:ANX2_HUMAN	P07355	Annexin II	19	2
sw:CBP2_HUMAN	P50454	Collagen-binding protein 2 precursor	20	2
sw:COF1_HUMAN	P23528	Cofilin, non-muscle isoform	21	<2
sw:CYPH_HUMAN	P05092	Peptidyl-prolyl cis-trans isomerase A	22	<2
sw:DYI2_HUMAN	Q13409	Dynein intermediate chain 2, cytosolic	23	2
sw:ECH1_HUMAN	Q13011	Delta3,5-Delta2,4-dienoyl-coa isomerase, mitochondrial precursor	24	2
sw:MLRN_HUMAN	P24844	Myosin regulatory light chain 2	48	2
sw:PLSL_HUMAN	P13796	L-Plastin	26	<2
sw:RAN_HUMAN	P17080	GTP-binding nuclear protein ran	27	3
sw:ROK_HUMAN	Q07244	Heterogeneous nuclear ribonucleoprotein k	28	2

sw:TCTP_HUMAN	P13693	Translationally controlled tumor	29	<2
sw:TPM1_HUMAN	P09493	Tropomyosin 1 alpha chain	30	<2
sw:TYPH_HUMAN	P19971	Thymidine phosphorylase precursor	31	5
sw:AMPL_HUMAN	P28838	Cytosol aminopeptidase	32	3
sw:K1CS_HUMAN	P08727	Keratin, type I cytoskeletal 19 (cytokeratin 19)	33	4
sw:ALDX_HUMAN	P14550	Alcohol dehydrogenase [NADP+]	34	4
sw:EL3A_HUMAN	P09093	Elastase IIIa precursor	35	4
sw:DLDH_HUMAN	P09622	Dihydrolipoamide dehydrogenase, mitochondrial precursor	36	2
sw:ECHM_HUMAN	P30084	Enoyl-CoA hydratase, mitochondrial precursor	37	3
sw:HSBX_HUMAN	O14558	Heat-shock 20 kDa like-protein p20.	38	2
sw:MLEN_HUMAN	P16475	Myosin light chain alkali, non-muscle isoform	39	3
sw:CALX_HUMAN	P27824	Calnexin precursor	40	3
sw:MA32_HUMAN	Q07021	Complement component 1	41	<2
sw:NUAM_HUMAN	P28331	NADH-ubiquinone oxidoreductase 75 kda subunit, mitochondrial precursor	42	2
sw:PBEF_HUMAN	P43490	Pre-B cell enhancing factor precursor.	43	2
sw:RET1_HUMAN	P09455	Retinol-binding protein I, cellular	44	2
sw:TCPG_HUMAN	P49368	T-complex protein 1, gamma subunit	45	2
sw:RINI_HUMAN	P13489	Placental ribonuclease inhibitor	46	<2
sw:GBLP_HUMAN	P25388	Guanine nucleotide-binding protein beta subunit-like protein 12.3	47	2
sw:S109_HUMAN	P06702	Calgranulin B	49	<2

Table 3: Proteins up-regulated in pancreatic cancer II

Protein	Acc No	Description	SEQ ID No	Fold Change
sw:CAPG_HUMAN	P40121	Macrophage capping protein	50	3
sw:ANX1_HUMAN	P04083	Annexin I (lipocortin I) (calpactin II)	51	4
sw:K2C7_HUMAN	P08729	Keratin, type II cytoskeletal 7	52	5
humangp:CHR13-Q15063	Q15063	Osteoblast specific factor 2 precursor	53	2
sw:TGLC_HUMAN	P21980	Protein-glutamine gamma-glutamyltransferase	54	2
sw:GDIR_HUMAN	P52565	Rho GDP-dissociation inhibitor 1	55	<2
sw:IQG1_HUMAN	P46940	Ras GTPase-activating-like protein	25	2

Table 4. Proteins roughly classified by their involved biological processing or basic function

Protein function[a]	Number[b]	Percentage (%)[C]
Structural constitural and regulation of cytoskeleton	61	20.8
Cell cycle and metabolism	74	25.3
Response to external stimulus or stress	61	20.8
Signal transduction	13	4.4
Nuclear function	18	6.1
Transport processing	19	6.5
Hemostatis	12	4.1
Cell adhension	7	2.4
Chaperon	7	2.4
Apoptosis	3	1
Unknown function	11	3.7
Others	7	2.4
Total	293	100

Table 5. Proteins with higher levels in normal pancreatic compared to cancer tissue

Protein[a]	AccNo [b]	Description[c]	Fold[d]	SEQ ID NO:
cytoskeletal regulation				
sw:DESM_HUMAN	P17661	desmin.	3	70
proteolysis and peptidolysis				
sw:CBPB_HUMAN	P15086	carboxypeptidase b precursor	2	71
sw:CBP1_HUMAN	P15085	carboxypeptidase a1 precursor	Uncalculated*	72
sw:CPB2_HUMAN	P50454	carboxypeptidase a2 precursor	Uncalculated*	73
sw:CTRB_HUMAN	P17538	chymotrypsinogen b precursor	Uncalculated*	74
sw:TRY1_HUMAN	P07477	trypsin I precursor (ec 3.4.21.4) (cationic	Uncalculated*	75

		trypsinogen).		
sw:TRY2_HUMAN	P07478	trypsin ii precursor (anionic trypsinogen).	3	76
sw:ILEU_HUMAN	P30740	leukocyte elastase inhibitor	4	77
chaperon				
sw:CH60_HUMAN	P10809	human. Mitochondrial matrix protein p1 precursor	2	78
sw:ENPL_HUMAN	P14625	94 kda glucose-regulated protein	3	79
SW:ER29_HUMAN	P30040	endoplasmic reticulum protein erp29 precursor	2	80
sw:PDA2_HUMAN	Q13087	protein disulfide isomerase a2 precursor	2	81
sw:PDA3_HUMAN	P30101	protein disulfide isomerase a3 precursor	2	82
oxidoreductase				
sw:ADHB_HUMAN	P00325	alcohol dehydrogenase beta chain	<2	83
sw:GTO1_HUMAN	P78417	glutathione transferase omega 1	Uncalculated*	84
sw:OXRP_HUMAN	Q9Y4L1	150 kda oxygen-regulated protein precursor	Uncalculated*	85
sw:PDX4_HUMAN	Q13162	peroxiredoxin 4	<2	86
sw:ULA4_HUMAN	P30039	mawd binding protein	<2	87
metabolism of biological process				
sw:AMYC_HUMAN	P19961	alpha-amylase 2b precursor	3	88
sw:AMYP_HUMAN	P04746	alpha-amylase, pancreatic precursor	Uncalculated*	89
sw:ATPA_HUMAN	P25705	atp synthase alpha chain, mitochondrial precursor	<2	90
sw:BAL_HUMAN	P19835	bile-salt-activated lipase precursor	3	91
sw:LIP1_HUMAN	P54315	pancreatic lipase related protein 1 precursor .	<2	92
sw:LIP2_HUMAN	P54317	pancreatic lipase related protein 2 precursor	Uncalculated*	93
sw:LIPP_HUMAN	P16233	triacylglycerol lipase, pancreatic precursor	Uncalculated*	94

sw:DPY2_HUMAN	Q16555	dihydropyrimidinase related protein-2		95
sw:GABT_HUMAN	P80404	4-aminobutyrate aminotransferase	2	96
sw:GATM_HUMAN	P50440	glycine amidinotransferase, mitochondrial precursor	Uncalculated*	97
sw:GR78_HUMAN	P11021	78 kda glucose-regulated protein precursor (grp 78)	3	98
sw:IF32_HUMAN	Q13347	eukaryotic translation initiation factor 3 subunit 2	<2	99
sw:DPY2_HUMAN	Q16555	dihydropyrimidinase related protein-2		100
sw:PGMU_HUMAN	P36871	phosphoglucomutase	2	101
sw:PSA1_HUMAN	P25786	proteasome subunit alpha type 1	2	102
heat shock protein				
sw:HS27_HUMAN	P04792	heat shock 27 kda protein	Uncalculated*	103
signaling				
SW:PD6I	Q8WUM4	programmed cell death 6 interacting protein	5	104
muscle development				
hsugp:057687-10-0	Q9bva2	four and a half lim domains 3	<2	105
SW:sli2	Q13643	Skeletal muscle LIM-protein 2 (SLIM 2)(Four and a half LIM domains protein 3)(FHL.3).	<2	106
transport of biological process				
sw:CYB5_HUMAN	P00167	cytochrome b5.	2	107
cell adhesion				
sw:PAP1_HUMAN	Q06141	pancreatitis-associated protein 1 precursor.	Uncalculated*	108
other				
SW:CA16_HUMAN	P12109	collagen alpha 1(vi) chain precursor.	5	109
SW:LUM_HUMAN	P51884	lumican precursor	3	110

Table 6 Proteins with higher levels in pancreatic cancer compared to in normal tissue

Protein [a]	AccNo[b]	Description[c]	Fold[d]	SEQ ID NO:
cytoskeletal regulation				
sw:FSC2_HUMAN	O14926	fascin 2 (retinal fascin).	2	56
sw:AAC1_HUMAN	P12814	alpha-actinin 1	2	7
sw:AAC4_HUMAN	O43707	alpha-actinin 4	2	5
sw:ABP2_HUMAN	P21333	endothelial actin-binding protein (alpha-filamin).	4	9
sw:ANX2_HUMAN	P07355	human annexin ii (lipocortin ii)	2	19
sw:CALD_HUMAN	Q05682	caldesmon (cdm).	2	12
sw:CAPB_HUMAN	P47756	f-actin capping protein beta subunit	5	15
sw:CAPG_HUMAN	P40121	macrophage capping protein	3	50
sw:COF1_HUMAN	P23528	cofilin, non-muscle isoform (p18).	<2	21
sw:DEST_HUMAN	P18282	destrin (actin-depolymerizing factor) (adf).	2	57
sw:DYI2_HUMAN	Q13409	dynein intermediate chain 2(fragment).	2	23
sw:GELS_HUMAN	P06396	gelsolin precursor	3	3
sw:K1CS_HUMAN	P08727	keratin, type I cytoskeletal 19	4	33
sw:K2C7_HUMAN	P08729	keratin, type ii cytoskeletal 7	5	52
sw:MLEN_HUMAN	P16475	myosin light chain alkali	3	39
sw:PLSL_HUMAN	P13796	l-plastin (lymphocyte cytosolic protein 1)	<2	26
sw:TAGL_HUMAN	P37802	transgelin (22 kda actin-binding protein).	2	10
sw:TBA4_HUMAN	P05215	tubulin alpha-4 chain.	2	8
proteolysis and peptidolysis				
sw:ACY1_HUMAN	Q03154	aminoacylase-1	5	14
sw:AMPL_HUMAN	P28838	cytosol aminopeptidase	3	32
sw:CATD_HUMAN	P07339	cathepsin d precursor.	<2	1
sw:CFAB_HUMAN	P00751	complement factor b precursor	5	4
sw:EL3A_HUMAN	P09093	elastase iiia precursor	4	35
chaperon				
sw:APE_HUMAN	P02649	apolipoprotein e precursor (apo-e).	<2	59
sw:CALX_HUMAN	P27824	calnexin precursor(p90)	3	40

sw:CYPH_HUMAN	P05092	peptidyl-prolyl cis-trans isomerase a	<2	22
sw:TCPG_HUMAN	P49368	t-complex protein 1, gamma subunit	2	45
sw:CBP2_HUMAN	P50454	human. Collagen-binding protein 2 precurso	2	20
tr_hum:Q96C61	Q96C61	hypothetical 88.6 kda protein	5	60
oxidoreductase				
sw:DLDH_HUMAN	P09622	dihydrolipoamide dehydrogenase	2	36
sw:IDHC_HUMAN	O75874	isocitrate dehydrogenase [nadp] cytoplasmic	2	2
sw:NUAM_HUMAN	P28331	nadh-ubiquinone oxidoreductase 75 kda subunit	2	42
metabolism of biological process				
sw:ALDX_HUMAN	P14550	alcohol dehydrogenase [nadp+]	4	34
sw:ECH1_HUMAN	Q13011	delta3,5-delta2,4-dienoyl-coa isomerase	2	24
sw:ECHM_HUMAN	P30084	enoyl-coa hydratase, mitochondrial precursor	3	37
sw:IPYR_HUMAN	Q15181	inorganic pyrophosphatase	<2	16
sw:TYPH_HUMAN	P19971	thymidine phosphorylase precursor	5	31
SW:ENOA_HUMAN	P06733	human alpha enolase	2	61
sw:ENOL_HUMAN	Q05524	alpha enolase, lung specific	2	13
sw:SYW_HUMAN	P23381	tryptophanyl-trna synthetase	2	62
heat shock protein				
sw:HSBX_HUMAN	O14558	heat-shock 20 kda like-protein p20.	2	38
signaling				
sw:GBLP_HUMAN	P25388	guanine nucleotide-binding protein beta subunit-like protein 12.3	2	47
sw:GDIR_HUMAN	P52565	rho gdp-dissociation inhibitor 1	<2	55
sw:IQG1_HUMAN	P46940	ras gtpase-activating-like protein iqgap1	2	25
sw:PBEF_HUMAN	P43490	pre-b cell enhancing factor precursor.	2	43
sw:RAN_HUMAN	P17080	gtp-binding nuclear protein ran	3	27
immune response				
sw:KAC_HUMAN	P01834	ig kappa chain c region.	2	63
sw:MA32_HUMAN	Q07021	pre-mrna splicing factor sf2, p32 subunit.	<2	41

inflammatory reponse				
sw:ANX1_HUMAN	P04083	annexin I (lipocortin i)	4	51
sw:LEG3_HUMAN	P17931	galectin-3	2	17
sw:S109_HUMAN	P06702	calgranulin b (mrp-14)	<2	49
muscle development				
sw:TPM1_HUMAN	P09493	tropomyosin 1 alpha chain	<2	30
sw:TPM4_HUMAN	P07226	tropomyosin alpha 4 chain	<2	11
sw:MLRN_HUMAN	P24844	myosin regulatory light chain 2	2	48
transport of biological process				
humangp:CHR2-Q15092	Q15092	transmembrane protein.	2	64
sw:POR2_HUMAN	P45880	voltage-dependent anion-selective channel protein 2	<2	18
sw:RET1_HUMAN	P09455	retinol-binding protein I	2	44
RNA processing				
humangp:CHR20-Q9P2E9	O75300	ribosome binding protein 1 (kiaa1398 protein).	<2	65
sw:RINI_HUMAN	P13489	placental ribonuclease inhibitor	<2	46
sw:ROK_HUMAN	Q07244	heterogeneous nuclear ribonucleoprotein k	2	28
blood coagulation				
sw:FIBG_HUMAN	P02679	fibrinogen gamma chain precursor	3	66
sw:THRB_HUMAN	P00734	prothrombin precursor	<2	67
Anti-apoptosis				
sw:TCTP_HUMAN	P13693	translationally controlled tumor protein (p23)	<2	29
cell adhesion				
humangp:CHR13-Q15063	Q15063	osteoblast specific factor 2 precursor	2	53
sw:BGH3_HUMAN	Q15582	transforming growth factor-beta induced protein	5	6
other				
sw:TGLC_HUMAN	P21980	tissue transglutaminase	2	54
sw:KPY1_HUMAN	P14618	Human pyruvate kinase, cytosolic thyroid hormone-binding protein	3	68
humangp:CHR19-Q96D15	Q96D15	Reticulocalbin 3 precursor.	2	69